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STUDIES IN THE PHYSIOLOGY AND MORPHOLOGY OF SOME CALIFORNIA HEPATICÆ.

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STUDIES IN THE PHYSIOLOGY AND MORPHOLOGY OF SOME CALIFORNIA HEPATICÆ.

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The morphology and physiology of the Hepaticæ have been treated by many authors, but their work has been confined mainly to the group as a whole. The intimate relations of the liverworts to their environments, have however, received only incidental treatment. Ordinarily we are inclined to associate with the Hepaticæ an environment characterized by moisture and shade. To a certain extent we are justified in doing so, for the majority of the known species occur in just such a habitat, many of the larger and more striking ones, such as *Monoclea*, *Dumortiera* and some species of *Aneura*, being common in the more humid regions of the tropics. On the other hand many species are known to occur normally in parts of the world where climatic conditions are not so evenly balanced as in the tropics; many thrive in extreme northern and southern regions where they are subject to great variations in temperature, while those growing in regions like the west coast of the United States must adapt themselves to prolonged periods of drought alternating with six or seven months of rainy weather.

It was with a view of ascertaining the nature and influence of these various conditions common to certain California hepaticæ that the present study was undertaken.

The author wishes to acknowledge his obligations to Professor Douglas H. Campbell and Associate Professor George J. Peirce under whose direction the work was pursued. Thanks are also due Professor Alexander W. Evans, Dr. Marshall A. Howe and Professor Roland Thaxter for assistance in the determination of material and for the use of certain hepaticæ sent to the writer.

PARASITISM AND SAPROPHYTISM IN HEPATICÆ.

The association of certain fungi with hepaticæ was first described in detail by Leitgeb,[1] who, in his studies on *Ptilidium ciliare*, observed the infection of young sporogonia. He found that all such sporogonia were more or less abnormal in their mode of segmentation and inferred from this that the infected organs were structurally effected by the action of the fungus.

Following Leitgeb, a number of writers have observed fungus infection in other hepaticæ. As early as 1879, Kny[2] discovered sterile fungal hyphæ in the rhizoids of *Lunularia* and *Marchantia*. These, he states, were found to be present in rhizoids undergoing a process of regeneration, which process may have been stimulated by the hyphæ. Cavers[3] has observed that when *Marchantia* and *Lunularia* grow in ordinary soil free of humus the rhizoids are penetrated by hyphæ which grow upward as delicate filaments showing cross-walls at rather long intervals. These hyphæ occasionally become branched but never, so far as he has observed, reach the tissue of the thallus. On the other hand, he has found that when these plants grow on humus soil the hyphæ extend into the compact tissue of the thallus, to which in fact, they are largely confined.

Golenkin[4] observed the presence of endotrophic mycorrhiza in *Marchantia palmata*, *M. paleacea*, *Preissia commutata*, *Tar-*

[1] Leitgeb: Untersuchungen über die Lebermoose, Heft 2, p. 58; Tafel 3, Fig. 26.

[2] Kny and Bottger, 1879: Ueber eigenthümliche Durchwachsungen an den Wurzelhaaren zweier Marchantiaceen. Verhandl. d. bot. Vereins d. Prov. Brandenburg, p. 2 of Separate.

[3] Cavers, 1903: Saprophytism and Mycorrhiza in Hepaticæ. The New Phytologist, Vol. II, No. 2, pp. 32–33.

[4] Golenkin, 1902: Die Mycorrhiza ähnlichen Bildungen der Marchanteen. Flora, Band 90, p. 209.

gionia hypophylla, *Plagiochasma elongatum* and *Fegatella conica*. In all these the hyphæ are confined to the more compact tissue of the thallus.

Jeffrey,[1] in his paper on the Gametophyte of *Botrychium virginianum*, describes an endophytic fungus associated with this fern. His observations show that conidia develop singly within the host cells usually at the end of a hypha. These peculiar conidia germinate *in situ*, generally producing a germ-tube which forces its way into the neighboring cells of the host. Within certain cells of the host he observed the development of vesicular structures along the enclosed hyphæ. Similar structures have been observed by Bruchmann[2] in his recent studies on *Ophioglossum vulgatum*. In his Fig. 42 he has indicated fungal structures similar to those common to the endophytic hyphæ found by the author in cells of *Aneura multifida major*.

The same writer[3] in his work upon *Botrychium lunaria* describes an endophytic fungus infecting the cells of the prothallium. He states that the hyphæ usually find entrance to the prothallial tissue through the rhizoids though in some instances they are found penetrating the outer, cuticularized surface of the prothallium. Once within the cells of the host, the hyphæ fill the cells with their sclerotia-like structures and *vesicular* enlargements. All starch of the infected cells disappears, but he finds oil and albumen within the hyphæ which he regards as reserve products to be utilized later by the growing embryo. Thus he recognizes a symbiotic relationship between fungus and host, and in another part of the same paper he advances the theory that the endophyte is a probable means in assisting the prothallium to withstand the rigors of hot and cold weather.

[1] Jeffrey, 1898: The Gametophyte of *Botrychium virginianum*. University of Toronto Studies, No. 1, p. 12.

[2] Bruchmann, 1904: Ueber das Prothallium und die Keimpflanze von *Ophioglossum vulgatum* L. Botanische Zeitung, Heft XII, Taf. VIII, Fig. 42 und Fig. 42*a*.

[3] Bruchmann, 1906: Ueber das Prothallium und die Sporenpflanze von *Botrychium lunaria* Sw. Flora, Band 96, Heft 1, pp. 210–211.

THE PARASITIC FUNGUS ASSOCIATED WITH FOSSOMBRONIA LONGISETA AUST.

In the writer's recent studies on the Development of *Fossombronia longiseta*[1] no reference was made to the structure of the infecting fungus; in fact, the material then in hand was too scanty to make anything like a satisfactory study. Since the publication of the above paper, however, excellent material has been found in various stages of development, and the relation of fungus to host has been clearly worked out.

So far as could be ascertained, the host is invaded from without by hyphæ that make their entrance through the rhizoids or directly through the cells of the host stem. The infection of the rhizoids is very similar to that described by Cavers[2] for *Lophozia*, *Cephalozia* and other hepaticæ. As a rule the hyphæ not only invade the rhizoids but extend throughout the tissues of the host, regardless of the nature of the substratum, thus differing somewhat from the nature of infection reported by Cavers where he has observed that the degree of infection varies with the amount of humus in the soil. In *Fossombronia*, as a rule, the hyphæ infecting rhizoids alone were confined to the inner wall of the organ, though in some instances not a few rhizoids were found in which hyphæ had penetrated through the wall to the exterior as shown in Fig. 3. The host thus affected was growing in soil containing little humus and in no case were these external hyphæ observed to grow longer than those figured. The hyphæ confined to the rhizoids are very seldom branched and are rather remotely septate. In certain parts, however, usually at the end of a branch, short segments not unlike conidia develop and are abstricted from the hypha. The subsequent behavior of these has not been observed. It is, however, quite probable that these structures furnish one means of transfer of infection. I did not find in *Fossombronia* anything comparable with the conidia described by Jeffrey[3] as occurring in the endophyte of *Botrychium virginianum*.

[1] Humphrey, 1906: The Development of *Fossombronia longiseta* Aust. Annals of Botany, Vol. XX, No. LXXVII.

[2] Loc. cit.

[3] Loc. cit.

In a few instances infected rhizoids were found to have undergone considerable modification of form as seen in Fig. 5. On the other hand very similar modifications were repeatedly observed in uninfected rhizoids. These were very likely due to the stimulus of contact with compact soil. Whether the fungus is instrumental in the development of such malformations as the one shown in Fig. 5 cannot be determined at this writing. It is, however, hardly probable, for the writer has observed that when infected plants are transferred to Knop's solution the new rhizoids though seriously infected maintain a uniform direction of growth and are apparently unaffected by the hyphæ.

In a previous paper[1] mention was made of the fact that *Fossombronia* plants in many instances developed a tuberous growth which, on careful examination, was found to contain a more or less complex growth of fungus hyphæ. Microchemical tests demonstrated the presence of a large quantity of starch, oil and nitrogenous food products within the cells of this tuberous growth and this, no doubt, in a measure accounts for the greater development of the fungus in this region. So far as the author has observed, none of these tuberous growths is free from infection. It is hardly probable, however, that this structure is directly due to the activity of the fungus for it is a structure common to a number of other hepaticæ such as *Geothallus tuberosus* Campb., *Riccia cancellata* Tayl., *Fossombronia tuberifera* Goebl., and a number of others in which no fungus infection has been observed. Specimens of *Geothallus tuberosus* have been carefully examined by the writer with a view to ascertaining the presence of infection, but in all material examined the tubers were uninfected and filled with reserve food products as described by Campbell,[2] '95. No doubt the tuber in the case of *Fossombronia longiseta* serves the same purpose as in *Geothallus tuberosus* or in *Fossombronia tuberifera*, *i. e.*, as a special structure to carry the plant through a more or less prolonged dry season. Howe[3] has shown that in specimens of

[1] Loc. cit.

[2] Campbell, 1896: A New California Liverwort. Botanical Gazette, Vol. 21, No. 1, p. 12.

[3] Howe, 1899: The Hepaticæ and Anthocerotes of California. Memoirs of the Torrey Botanical Club, Vol. VII, p. 80.

Fossombronia longiseta collected in southern California where the dry season is longer, this tuberous growth is considerably more marked than in the case of plants in the northern half of the state. Owing to the earthquake of April 18, 1906, a break occurred in a water-pipe not far from Stanford University. As a consequence a considerable tract of ground was well irrigated throughout the past, very prolonged dry summer. Upon this thoroughly moistened soil the writer found, growing vigorously, a considerable number of plants of *Fossombronia longiseta*, *Anthoceros pearsoni*, *Targionia hypophylla*, and some species of the more common mosses. Here were growing a number of plants accustomed to summer desiccation and it occurred to the writer that under these conditions of increased humidity certain structural changes might result both in the development of the gametophyte and sporophyte.

Careful examination of a large number of these plants revealed no evidence of anything in the nature of a tuberous growth in any part of the thallus though all plants examined showed fungus infection. It would seem therefore that this instance would lend some support to the inference that, so far as our species of *Fossombronia* is concerned, these small tuber-like structures are purely adaptive and their development depends largely upon certain external factors. Whether growth under similar conditions would result in the reduction or disappearance of the tubers in such plants as *Geothallus tuberosus* or *Anthoceros phymatodes* is a matter that has not yet been tested. In these forms the tuber is a well-marked and doubtless long-established modification of the thallus and if reduced at all would probably require a considerable period of time during which conditions of constant moisture are allowed to act. Peirce,[1] '06, has demonstrated that such a well-marked character as the dorsiventrality of certain liverwort gametophytes is not a hereditary character as commonly supposed but is primarily due to the formative influence of light.

As elsewhere stated, some plants of *Fossombronia longiseta* were studied in which the rhizoids were apparently free from

[1] Peirce, 1906: Studies of Irritability in Plants. Annals of Botany, Vol. XX, No. LXXX, p. 459.

hyphæ, though the more compact tissue of the thallus was in many such instances thoroughly infected. Thus it would seem that infection need not necessarily take place through the rhizoids. Material carefully fixed in chromic acid (1 per cent.) and stained with the Fleming's triple combination seemed to give very satisfactory results, though it was found necessary to allow more time to the action of the stain than was customary in staining organs or structures of the host. Sections through the stem of infected plants almost always showed a well-defined zone of cells more or less given over to hyphæ. This is not unlike the condition observed by Cavers[1] ('03) in *Fegatella* and by Golenkin[2] ('02) in a number of forms cited above. Golenkin states that the cells of this zone, though still retaining their protoplasm and nuclei, are void of chlorophyll and starch. Careful examination of infected cells of *Fossombronia*, except where infection was so far advanced that the cells were occupied by developing sclerotia, revealed the presence of varying amounts of chlorophyll and starch, depending upon the number and vigor of invading hyphæ.

Unlike the forms described by Golenkin, this fungus is not necessarily confined to any given zone, for any portion of the thallus may be invaded by hyphæ extending out from the more compact parts. Though several hundred fruiting plants have been studied, no evidence of infection of either antheridium or archegonium has yet been seen, and the sporophyte is apparently free from the attack by fungus.

In material fixed in January, 1904, about three months after the beginning of the rainy season, almost the entire tissue of the stem was packed with hyphæ, many branches extending through the outer layer of cells of the host into the substratum.

Although the fungus was present in virtually the whole of the stem-tissue, strictly speaking, it was found that at this stage of its development the hyphæ were more or less confined to the innermost cells of the stem while those in proximity to the growing point as well as the leaves were apparently free from infection. The epidermal cells and those immediately beneath

[1] Loc. cit., p. 33.
[2] Loc. cit., p. 209.

showed evidences of comparatively few hyphæ which evidently served to connect the more functionally active interior system with those in the rhizoids and with the substratum. Thus far the habit of growth of this fungus agrees quite consistently with those described by Cavers.[1]

At about this period in its development one may observe, scattered here and there throughout the infected zone, vesicles of considerable size and presenting a variety of form. These appear as great enlargements of the hyphæ and are full of rather coarsely granular protoplasm (Fig. 6). This protoplasm has within it minute nuclei, at first rather few in number but as the vesicles become older and larger increasing in number until they quite fill the interior of the vesicle. It was further found that in some instances at least these vesicles are connected with the exterior by hyphæ (Fig. 8) and along the course of these hyphæ may be seen certain of these nuclear bodies. Whether or not they make their escape to the exterior as zoöspores could not be demonstrated, but their development and subsequent behavior is suggestive. In his recent work upon the development of *Ophioglossum pendulum* and other members of the genus, Campbell[2] has demonstrated the presence of thin-walled vesicles in the endophyte. In these the behavior of the nuclei resembles that occurring in the vesicles of the endophytic fungus found in the *Fossombronia*. In *Fossombronia* these vesicles completely fill the host cells in which they occur, in some cases causing a considerable distention of the cell.

As stated above, the amount of chlorophyll or of starch and other products of metabolism varies with the degree of infection. Certain cells of the host seem packed with hyphæ, and a microchemical test fails to reveal any of the products of the normal cell, and while a large part of the stem appears to be dying, the leaves and the uninfected stem tissue seem to be vigorous and unaffected by the presence of the fungus. Comparison of infected plants with those entirely or nearly free from fungus invasion seems to demonstrate that up to the vesicle stage of development the growth of the host is not abnormally stimulated, in

[1] Loc. cit., p. 32–33.

[2] Campbell, 1907.

fact an extensive examination of plants of both sorts leads one to conclude that the presence of the fungus is of no advantage to the host and in many instances is an evident detriment. In March, 1906, while making some field studies upon *Fossombronia*, the writer observed that certain plants appeared to be dying. The affected plants were in some instances almost white, apparently from loss of chlorophyll. Closer examination with a hand-lens revealed the presence of a great number of minute black bodies along the length of the stem and in the leaves. These were especially abundant near the base of the leaf. On further examination with the microscope it was found that these black bodies were sclerotia within the cells of the host and the development of these resulted ultimately in the death of the host. It was at first thought probable that this fungus was distinct from the one found invading the rhizoids and stem tissue, but a careful study seems to show that the two are identical. At least so far as concerns the host the development of these sclerotia marks the period of greatest activity of the fungus, and its truly parasitic nature is manifested. Up to the appearance of the sclerotia the life of the host is not seriously affected but as soon as the hyphæ begin to extend and to form the structures that later develop sclerotia a very evident drain upon the vitality of the host sets in and increases with the further development of these structures.

It was at first thought that these might be perithecia of some ascomycete but microtome sections demonstrated their true nature. Wherever they occur the cells containing them become considerably distended and completely filled. Their presence causes no abnormal development of the cell as regards thickness of wall or form, though the cell-contents become much modified to the extent that all vestige of any starch, oil, cytoplasm, etc., has disappeared, even the nucleus is absorbed and the entire cell cavity is filled by the sclerotium.

The behavior of the hyphæ with reference to the nucleus and chromatophores as described by Cavers[1] for the fungus infecting *Monoclea* does not occur in the case of the fungus under

[1] Cavers, 1904 : Contributions to the Biology of the Hepaticæ. Part I. *Targionia*, *Reboulia*, *Preissia*, *Monoclea*, p. 39.

consideration. In *Monoclea* the fungus is confined to a sharply defined mycorrhizal zone three or four cell layers in thickness. Many of the cells in this zone are filled with branched hyphæ, tufts of which seem to envelop the nucleus. In some cases even the chromatophores, like the nucleus, become surrounded by similar tufts of branching hyphæ in a manner quite suggesting the formation of a lichen.

In *Fossombronia* the hyphæ preceding the development of a sclerotium are but sparsely ramified. The first indication of the sclerotium is the increase in diameter of the hyphæ within a cell followed later by the profuse development of short, thick anastomosing branches between which may at first be seen spaces of varying size which ultimately disappear as the sclerotium increases in extent (Fig. 10). The nucleus and chromatophores at first visible finally disappear, doubtless being taken up by the actively growing fungus. As yet the writer has failed to observe how these structures are consumed.

According to Czapek[1] the tissues of *Marchantia*, *Fegatella*, *Lunularia* and other hepaticæ contain an antiseptic principle which he calls "sphagnol" because of its abundance in the peat-mosses. He has shown that this substance exists in combination with the cellulose of the cell-walls and exerts an inhibitive influence upon the development of moulds and bacteria. This, Cavers[2] thinks, suggests the view that in the case of certain Fusarium-like fungi the "sphagnol" may serve to regulate the growth of the fungus and prevent symbiosis from passing into parasitism. In the case of *Fossombronia* it cannot be shown that at any time during a period of infection the invading fungus maintains a symbiotic relation with reference to the host, for an examination of infected cells shows that the presence of hyphæ sets up a disturbance of the cell-metabolism, the cell becoming impaired to such an extent as ultimately to cause its death. The degree of this impairment varies with the activity and extent of the parasite. To be sure, many infected plants appear to be quite as healthy and fertile as

[1] Czapek, 1889: Zur Chemie der Zell membranen bei den Laub. und Leber-moosen. Flora, Band 86, p. 361.

[2] Loc. cit., p. 33.

others yet free from the fungus, but it is owing to the fact that the degree of infection is but slight and the vigor of the host is sufficient to throw off for a time the harmful effects of the parasite just as the leaves of cultivated lettuce maintain apparent full vigor during early stages of the infection by *Bremia lactuca*.[1]

Experiments designed to ascertain the effect of the fungus upon very young plants of either *Fossombronia* or *Fimbriaria* have thus far failed, and it cannot be decisively stated here that *Fimbriaria* is at all susceptible to infection by this fungus. The examination of a large number of plants of all stages of development has shown our common species, *F. californica*, comparatively free from infection by any fungus. Recently, however, the author has found a few plants of this species attacked by a fungus which seems, in material thus far examined, to be confined entirely to the cells of the first four layers on the ventral side of the thallus. Hyphæ in considerable numbers were observed in both the smooth and tuberculate rhizoids. These showed but slight tendency toward branching, in many instances extending the entire length of a rhizoid without producing a branch. The hyphæ confined to the rhizoids, compared with those within the mycorrhizal zone of the thallus, are very much more delicate; but upon extending into the cells of the thallus they branch profusely and develop relatively thick and tough walls. The fungus resembles the one described above for *Fossombronia*, in that it is filamentous and septate. Structures comparable to vesicles, conidia or sclerotia have not yet been observed. The presence of the fungus seems in no wise to retard the growth or affect the vigor of the thallus and no plants have yet been found in which the hyphæ have penetrated the chlorophyll-bearing tissue.

THE PARASITIC FUNGUS ASSOCIATED WITH ANEURA MULTIFIDA MAJOR.

While in the field collecting *Aneura* the writer observed that a considerable number of plants had taken on a rusty brown

[1] An illuminating discussion of symbiosis and parasitism is to be found in Plant Physiology. Peirce. Pp. 85–92 inclusive.

color. Upon careful examination it was found that they were in a dying condition owing to the presence of a fungus. A large quantity of material consisting of plants in a normal condition as well as those visibly affected by the fungus were brought into the laboratory in September, 1906, where the relation of fungus to host could be more carefully studied. This species of *Aneura* is found growing on moist surfaces of rocks along streams, on decaying logs and moist banks of soil in which there is considerable clay. Material collected from all three of these sources contained a large number of infected plants so it is hardly probable that the fungus is one confined to plants growing on decaying wood.

Aneura multifida major, as well as other species of this genus, under certain conditions produce two-celled gemmæ in great numbers. It was found that many of these after a short time had germinated and some were infected. Figure 15 shows a young plant resulting from the germination of a gemma, into the older cells of which a fungus had penetrated by well-marked haustoria. The diseased cells contained less chlorophyll and showed unmistakable evidences of the harmful effect of the fungus. Young plants of varying age and size were found to be infected. It was first thought likely that infection took place directly while the gemma was still within its mother cell, but a very careful examination of gemmæ failed to support any such view. It seems that young plants of less than four cells are rarely infected. The fungus develops conidia freely and it is probable that infection is brought about by their germination. A small number of young plants developing from germinating spores were observed, but as yet none of these has shown the presence of a fungus.

In the older plants, hyphæ from within the cells of the thallus grow downward extending into the rhizoids, though this is by no means so common as in the case of *Fossombronia* or *Fimbriaria*. Many rhizoids whether infected or not present a strong tendency to branch as shown in Fig. 16, *a* and *b*. This is undoubtedly due to the influence of contact stimulus; in fact, rhizoids not in contact with the substratum are all quite simple and more delicate.

In his studies upon *Lophocolea bidentata*, Cavers[1] found that the tissues of the gametophyte are entirely free from hyphæ, but the rhizoids which grow out in tufts from the bases of the amphigastria penetrate the substratum of rotten wood and there become profusely branched like the haustoria of many fungi. This, he considers, enables the liverwort to assume a more or less saprophytic existence. He has not however shown that the plant actually does adapt itself to this mode of life, nor does it appear that we can assume the branching of the rhizoids as due to anything other than contact stimulus. Peirce and Randolph[2] have demonstrated that in the case of certain attached fresh-water algæ and in many marine forms the development of a holdfast is directly and wholly the result of contact stimulus. The complexity and extent of the holdfast were found to vary with the degree of roughness of the surface of the substratum; young plants grown on ground glass developing much more elaborate holdfasts than similar plants grown on smooth glass while those grown in dust-free water developed no holdfasts at all.

In Knop's solution the writer now has plants of *Fossombronia*, *Cryptomitrium* and other liverworts growing that were removed from a normal soil substratum some months ago. Still attached to these plants are some of the old rhizoids which are all more or less gnarled and modified, some exhibiting short lateral processes. Since placing the plants in Knop's solution a great number of rhizoids have developed and in every instance they are perfectly straight and unmodified and much more delicate in structure than those that had grown while the plants were in their normal habitat. We have here exactly the same behavior exhibited by the secondary roots and root-hairs of higher plants. If, as some botanists maintain, the rhizoids of bryophytes are simply organs of attachment is it not probable that, as in the case of certain algæ, these branches are due to the stimulus afforded by contact?

Aside from *Aneura* and *Fossombronia* the writer has observed branching rhizoids in *Cephalozia bicuspidata*, due probably to

[1] Loc. cit., p. 32.

[2] Peirce and Randolph, 1905: Studies of Irritability in Algæ. Botanical Gazette, 40 · pp. 321–350.

the same stimulus, as they were not infected by hyphæ. The fungus associated with *Aneura* differs from that attacking *Fossombronia* both as to development and habit of growth. In the case of the *Fossombronia* fungus we find it to be more truly endophytic, once infection has been brought about, while the fungus associated with *Aneura* seems to be epiphytic in habit, developing haustoria-like branches which in turn develop other branches penetrating and drawing sustenance from the host cells. The hyphæ are septate and profusely branched, especially within the tissues of the host. The hyphæ within the rhizoids are in nearly every instance unbranched and in no case were they seen to grow through the wall of the rhizoid communicating with the exterior as in *Fossombronia*.

The physiological effect of the fungus upon the host is quite as marked as any we have yet studied. The cell walls of *Aneura* are very much thicker than those of most of our liverworts, and the plants are vigorous in habit. Each cell contains usually one large oil body and numerous chromatophores. Fungal hyphæ penetrate the cell walls without any resulting modification and in some instances a single branch may pass through several cells without producing haustoria or branches of any sort. Ordinarily, however, after entrance to the host tissues is effected, the fungus becomes quite extensive, as seen in Fig. 13, where three affected cells are represented. In these it will be observed that the large oil body has already disappeared and the number of chromatophores is somewhat below the normal (compare Fig. 12 with Fig. 14). Ultimately the cells become in some instances packed with hyphæ and at this stage the death of the cell rapidly ensues. Such cells when microchemically tested are void of starch, no nucleus or cytoplasm can be made out, and the chromatophores have undergone complete disorganization; in fact, little remains but the cell wall. In certain plants where the fungus had reached an advanced stage the hyphæ within certain cells had developed into knot-like structures suggesting the beginning of sclerotia; and in a few instances these had, when sufficiently nourished, developed into blackish thick-walled sclerotia completely filling and distending the cell cavity. These, in appearance and structure, very much

resemble the sclerotia formed in the cells of *Fossombronia*, though they do not develop in such great numbers. This is doubtless due to the fact that the comparatively early decline and death of the host tissues impairs the vigor and health of the parasite before these structures can develop in great numbers.

As soon as the host cells begin to show the effects of parasitism there develop upon the surface of the host at indefinite points along the surface-hyphæ, perithecia-like structures (Fig. 11) the real function of which, however, has not been demonstrated. Professor Roland Thaxter kindly attempted to identify the fungus for me but being unable to determine the true nature of these structures was forced to wait for more advanced stages. The writer has prepared a number of slides of microtome sections which show these bodies to be hollow.

Beyond a wall no other structures have yet been observed in connection with the interior of these somewhat globular bodies. They seem to develop from short processes that appear here and there along the external hyphæ. They assume very early the spherical habit and develop at various points on their surface short spine-like processes at first pointing in different directions but ultimately either disappearing or lying all in one plane at the base of the structure bearing them. On certain host plants the tissues of which have already begun to turn brown, these perithecia-like bodies develop in such numbers as to be almost in contact, and the complex of hyphæ on the surface and within the host is very elaborate. Two-celled conidia were found on the surface of the host but it could not be proved that these spores belonged to the fungus infecting *Aneura*, although no other fungus was observed in the material.

It is the intention of the writer to work out the development and life history of this fungus and the one associated with *Fossombronia* more fully in a later paper. At the present moment the main object is to discuss the relation of parasite and host. In the case of this fungus there is less evidence of anything comparable to a symbiotic relation than in the relation of the *Fossombronia* parasite to its host. In fact, as soon as infection is brought about, the host seems to show signs of resulting

injury. The infection spreads from plant to plant quite rapidly. In a dinner-plate full of material, at first the majority of plants examined were unaccompanied by fungus, but within three weeks, on one side of the plate all the plants were turning brown; on examining these they were found to be infected. The fungus seems usually to make its attack through the ventral side of the thallus, though occasionally a plant would be found with hyphæ on both sides. Microtome sections failed to reveal any vesicular structures within the host.

Aneura, being naturally less compact and vigorous than such forms as *Fegatalla* or *Lunularia*, furnishes a poorer field for a parasitic fungus and seems less able to cope with the fungus. This, however, may be partly due to greater vigor of the parasite. It must also be noted that infected plants behave differently under varying conditions. *Aneura multifida major* as we find it here is semi-aquatic as to habitat and when growing under perfectly normal conditions may be considerably infected without showing any effect other than becoming yellowish green in color. If, however, these same plants are brought into the laboratory, even though well supplied with moisture, under a bell-jar they become brown in color finally dying, while uninfected plants seem to thrive quite as well as when growing out-of-doors under normal conditions. Plants growing along streams, though receiving little less than the required amount of water, if infected, seem less able to throw off the effects of the fungus than plants growing under perfectly normal conditions of moisture, light, etc.

Aside from the fungi found in association with *Fossombronia*, *Fimbriaria* and *Aneura*, the writer has had occasion to study infected material of *Anthoceros pearsoni* and *Porella bolanderi*. In habit of growth the fungus associated with *Anthoceros* is very much like that infecting *Aneura* though none of the material examined showed any structures suggestive of fruiting organs other than conidia which were being formed in considerable numbers by abstriction along the external hyphæ. They branch freely and are seen to anastomose not at single points here and there but continuously for some length, both hyphæ being rather minutely septate along the length of contact. The

hyphæ produce short lateral processes which grow in length until they come in contact with a similar branch of a neighboring hypha effecting conjugation with it; or in case it fails to meet with such a branch it is seen to anastomose with a nearby hypha and in this manner a complicated weft of hyphæ may develop over the surface of the host. The same seems to be true with regard to the hyphæ within the host cells, though to less extent. Within certain cells (Fig. 19) dark colored sclerotia-like structures develop, though in the material studied these were by no means abundant. With a limited amount of infected material nothing definite can be said as to the relation of the fungus to the host. All plants examined however seemed practically unaffected by the fungus. Infected cells exhibited the single chromatophore in every case except where sclerotia had formed. Just what would have been the effect had the fungus been as far advanced as in the case of *Fossombronia* or *Aneura* is mere conjecture. Our species of *Anthoceros* have, growing within the thallus, colonies of a species of *Nostoc*, but the writer saw no evidence of fungal hyphæ invading these.

Porella bolanderi is infected by a fungus of somewhat different habit, though its ultimate effect upon the host is not greatly different from what has been described for *Fossombronia* and *Aneura*.

The principal hyphal trunks appear for the most part to be intercellular, thus differing in this respect from the others described above. A single hypha finding entrance to a leaf for example, will ramify to some extent, and these various primary branches grow along between the cells of the host, thus forming an intercellular complex that may effect nearly every cell. From these primary branches, haustoria find entrance to the host cells and here they become more or less branched, with the result that the cells lose all their contents except the nucleus; this seems to persist. After the disorganization and disappearance of the chromatophores, the fungus structures are easily visible and in many of these cells may be seen exceedingly thick-walled chlamydospores, in some instances quite filling the enclosing wall of the host cell (Fig. 17).

The fungus is not confined wholly to the interior of the tissue.

In many cases a complex of hyphæ over-runs the surface of a leaf and it is not uncommon to find chlamydospores in connection with these external hyphæ. One-celled conidia are abstricted in large numbers from branches of the external hyphæ. Sclerotia have not yet been found, though occasionally a cell may be seen well filled with knots of hyphæ, too loose in structure, however, to be regarded as sclerotia. Cells in which these knots occur are always dead and apparently empty.

The fungus behaves toward the host as a parasite. Its hyphæ produce haustoria in great numbers which in the course of time invade all the cells of a leaf, thus impairing greatly its ability to elaborate food materials. The younger leaves of the host are apparently uninfected, and even though the older parts of the thallus may be dead, growth continues at the growing point and the plant may grow indefinitely, though very evidently diseased.

The writer has not thus far observed a fungus associated with our species of *Sphærocarpus* or with *Targionia hypophylla*, although Golenkin[1] reports the existence of an endotrophic mycorrhiza for the latter, and it may be that further investigation will demonstrate the presence of a fungus in *Targionia* as we find it here. As a matter of fact it is not improbable that all the hepaticæ of this region may on further study prove to be infected, the fungus being either a symbiont or, what is more likely, a parasite. The writer has seen no evidence in favor of the view that the fungus stimulates the growth or in any way benefits the host, though the presence of hyphæ may lead up to the development of abnormal structures such as modified root-hairs, enlarged cells, etc.

Bruchmann,[2] in his studies on *Botrychium lunaria*, concludes as follows: "Der physiologische Nutzen des Zusammenlebens von Prothallium und Pilz scheint mir auch hier nicht darin zu bestehen, dass die wenigen und unscheinbaren Pilzfaden, welche aus dem Substrat des Gamophyten in ihn emmünden, belanglose Humusstoffe ihm zuführen, Sondern nur darin, dass die von den functionsfähigen Rhizoiden der wachsenden Schutelpartie aufgelösten und herbeigeführten Baustoffe mit-

[1] Loc. cit., p. 209.

[2] Loc. cit.

telst des Stoffwechsels des Endophyten in haltbare Reservestoffe umgewandelt und aufgespeichert werden, von denen namentlich die auffallend grosse Oelmenge dem Prothallium die Fähigkeit verleiht, in dem sandigen Boden auch während der Sommerhitze und der Winterkälte von Austrocknung bewahrt zu bleiben."

As already stated, the author has found, in the case of all infected forms thus far investigated, oil and of necessity albumen, in the living cells of the gametophyte. In *Fossombronia* and *Aneura* at a certain stage in the development of the endophyte there is a minimum quantity of these products within the host cells containing hyphæ and a maximum amount of these same products within the cells of the fungus. The result is the death of the host or, at least, a very serious check to its development, resulting in small sickly plants. In such a case we cannot possibly regard the food products derived from the gametophyte as reserve materials later to be given up by the fungus as nourishment to further growth of the host.

The only forms examined not showing harmful effects from association with a fungus were *Fimbriaria*, *Fegatella* and *Anthoceros*. Of the first and the last, only a very limited amount of infected material was found, consequently little light can be thrown upon actual relation of host to endophyte. Howe[1] has reported an endophytic fungus associated with *Anthoceros olneyi* Aust., the septate hyphæ of which produce at the ends of lateral branches, globular clusters within which are produced numerous dark spore-like cells bearing some resemblance to those of the Tilletiaceæ. He has found the same parasite in *A. ravenellii* Ala. (Mohr.) and the same or one very similar in *A. hallii* Aus. He regards this fungus as a parasite, though no detailed account is given relative to the character of the association of the two.

DEVELOPMENT OF FRUITING ORGANS OF FEGATELLA CONICA IN CALIFORNIA.

Fegatella conica (*Conocephalum conicum*) is described by Howe[2] as growing in moist, deeply shaded places, especially

[1] Howe, 1898: The Anthocerotaceæ of North America, Bulletin of the Torrey Botanical Club, Vol. 25, No. 1.

[2] Loc. cit., p. 58.

on stones and rocks beside streams. It is not common in California and was first collected by Professor Underwood at Felton, Santa Cruz County, in 1888. In July, 1906, it was found in fruiting condition by the author on a trip up San José Cañon in the Santa Lucia Mountains in Monterey County. So far as is known, plants bearing receptacles had not been found in California prior to the above named date, though fruiting plants have been collected by C. V. Piper near Seattle, Wash. Conditions during the rainy season there and in the parts of California where *Fegatella* thrives are similar. The dry season near Puget Sound is occasionally interrupted by summer showers which may in combination with other factors influence the development of fruiting organs.

Fegatella may be found in many of our near-by cañons; in fact, it has been observed to occur along the banks of creeks well down upon the plain of the Santa Clara valley, but almost always it will be found in particularly well watered and densely shaded localities, a combination of conditions very essential to the normal development of the plant in this region of long, dry summers.

Antheridial plants bearing receptacles have been collected from several localities, though these plants are by no means common. Normally, they are of such vigorous, robust habit as to spread over a considerable area by mere vegetative growth, and it is not uncommon to find several square feet entirely covered by *Fegatella* to the exclusion of everything else.

On finding female plants in fertile condition a field study was made relative to their habitat, and conditions affecting growth and development of fruiting organs. San José Cañon is a deep, narrow gorge, the floor of which is well watered by a never-failing stream. The north wall of the cañon consists very largely of precipitous rock ledges that in many places come abruptly to the stream's edge and are only sparsely clothed with *Artemesia*, *Adenostoma* and a few other plants of xerophytic habit. The south wall rises less abruptly into hills averaging seven to eight hundred feet in height. These have a covering of rich humus and the vegetation at the base along the creek and well up on the south slope consists of *Sequoia*

sempervirens, *Alnus rhombifolia*, *Salix lasiolepis* and other trees which afford abundant shelter for a variety of shade-loving plants. Here also thrive several species of mosses and liverworts that in less favored localities would either die or pass into a dormant, air-dry condition.

Fegatella occurs throughout the length of the cañon on both banks of the creek, though most abundantly on the south side where there is a minimum of direct sunlight and evaporation. Five trips were made during the past season for at least a distance of three miles toward the head of the cañon and each time a diligent search was made for fruiting plants which were found in but few, very limited areas, where the exposure was north or northeast. It was observed that no fruiting plants were to be found in places void of sunlight, and the same was true when the plants grew within six or eight inches of the water's surface in light of the same intensity as fell upon nearby fruiting plants. Plants bearing receptacles in greatest number were found on a flat elevation about eighteen inches above the water in a break between trees through which fell a flood of strong, diffused light and at midday direct sunlight. This level bed of *Fegatella* was bathed constantly by seepage of spring-water from a moss and fern grown cliff forming the background, upon which were found a few fruiting plants of *Fegatella* and *Aneura pinguis*. Wherever fruiting plants occurred, it was noted that the combined conditions of light and moisture were relatively about the same. In places where there was strong light but insufficient moisture the plants were observed to wither, and when these conditions were reversed there was always a strikingly vigorous vegetative development, some individual plants measuring ten or more inches in length.

The time of fertilization of our California plants is not definitely known. The antherozoids are discharged during March and April and receptacles still containing intact antheridia have been found in some of our cañons as late as August.

In England, according to Cavers,[1] the receptacles begin to develop in early spring but do not mature until about the end of June, while the earliest stages in the development of the sporo-

[1] Loc. cit., p. 98.

gonium are observed about the middle of July. Owing to the very slow development of the sporogonium the spores are not discharged until the following spring. Here in California our plants behave differently. After fertilization the female receptacles grow to about two to two and one half millimeters in diameter. Though the plants may be amply supplied with moisture from below throughout the summer, proper humidity and temperature conditions do not prevail until the beginning of the winter or rainy season. Throughout the summer the growth of the receptacle is so slight as to be almost imperceptible. San José Creek empties into Carmel Bay and in late summer and autumn, the cañon, being near the sea, is quite commonly filled with heavy fogs for a part of the day, leaving all vegetation dripping wet. While these fogs are not equivalent to the drenching rains of winter, they do play a great rôle in the revival of certain mosses, liverworts and lichens and doubtless stimulate their growth to a certain extent. At any rate, up to the latter part of August the archegonial receptacle of *Fegatella* increases but slightly in size, though the increase is sufficient to enable one to distinguish fertilized from unfertilized receptacles. By the first of October (before any rain had fallen) the sporogonium had advanced to the spore tetrad stage and remained in about this condition until the opening of the rainy season. Following this, rapid development ensues and the spores are discharged in January.

This liverwort might be considered as viviparous, since the spores germinate within the capsule. Cavers states that germination occurs within the capsule before the spores are discharged, but does not say how long a time elapses between the first evidences of germination, and the ultimate elongation of the peduncle and dehiscence of the capsule. With our plants germination-stages were found at least a month preceding the liberation of the spores. How much earlier than this germination actually begins cannot be said, as material for examination was not available at that time. Germination does not take place simultaneously in all the spores of a single capsule. One may find within a capsule every stage from the ungerminated spore to the six-cell stage, and it is to be

noted that a certain percentage of spores do not germinate at all, but may be seen in a collapsed and shrunken condition within the capsule.

Most of the plants examined were infected by a mycorrhizal fungus that agrees closely in structure and habit with the one described by Cavers. Our observations confirm this as to the presence of starch within infected cells which is contrary to the view of Golenkin,[1] who investigated a number of infected hepaticæ, including Fegatella, and states that infected cells contain no starch or chloroplasts.

Our material was found growing on sandy soil containing considerable humus and it was found that but few rhizoids contained hyphæ. No plants were observed in which the ventral tissue of the midrib was infected to any extent as shown by Cavers in plants collected near the sewer-like drains of tanneries and similar works. All infected plants were in vigorous, healthy, growing condition and so far as could be ascertained, the presence of a fungus entails no such disastrous effects as one may observe in more delicate forms such as *Fossombronia* or *Aneura*.

ADAPTATION OF CERTAIN LIVERWORTS TO THE DRY SEASON.

It is commonly assumed that bryophytes are, in general, moisture-loving plants. While this is to a great extent true it may easily be shown that there are many important exceptions. Among the mosses we have only to note such forms as *Grimmia*, *Hedwegia*, *Andreœa* and many others common to different parts of the world growing on exposed rock surfaces, with ability to revive promptly and resume growth after the first rain. Likewise many foliose Jungermanniaceæ, common in the eastern part of the United States, are capable of withstanding drought periods of considerable length, lying practically dormant throughout the rainless interval. To what extent eastern thallose Jungermanniaceæ are capable of withstanding drought is a question, that so far as the writer is aware, has not yet been investigated.

In this section of California, and more particularly the low-

[1] Loc. cit., pp. 209–220.

lying country about San Francisco Bay there is an interesting hepatic flora consisting of types of most of the principal groups. All of these forms fruit luxuriantly and are seldom found sterile during any one growing season.

Among the more common hepaticæ of this region, and especially in the vicinity of Stanford University, are certain species of *Riccia*, including *R. glauca* and *R. trichocarpa*, both of which grow vigorously in localities of extreme exposure; and especially is this true of the latter, a liverwort structurally adapted to such a habitat. Aside from several species of *Riccia* two species of *Fimbriaria* (*Asterella*), *F. californica* and *F. violacea* are met with, the former being quite common. In certain localities, especially along the high banks of arroyos, *Cryptomitrium tenerum* is found to be abundant. Along with it and in places of severer exposure, occur formations consisting almost exclusively of the highly resistant *Targionia hypophylla*.

Along the banks of our coast-range streams, even where these come down into the Santa Clara valley, occur such widely distributed forms as *Fegatella conica* and *Marchantia polymorpha*. These however always grow in close proximity to the water where throughout the year they may never be entirely deprived of a supply of moisture. It is doubtful if these forms would be capable of withstanding the effects of a dry season. Three years ago the writer transferred several plants of *Fegatella* to a locality in the valley where conditions of light and substratum were restored as nearly as could be done, at the same time modifying and finally cutting off altogether the supply of moisture. By lessening for a whole summer season the supply of moisture it was found that growth of the thallus was greatly checked and the resulting branches were much shorter. Plants that were deprived of water dried up and died, all ability to recover being lost as soon as the tissue had given up a certain amount of the water of constitution. It cannot here be said to what extent *Marchantia polymorpha* is capable of withstanding drought, but since it is almost never found except where there is a fairly constant supply of moisture it would probably endure desiccation little better than *Fegatella conica*. Other moisture-loving liverworts found in moist shaded cañons of the outer

coast ranges are two species of *Aneura* (*Riccardia*), *A. multifida major* and *A. pinguis*, the latter being far more sensitive to change of environment than the former, but neither is capable of withstanding much loss of water. They are both found growing at their best when, if not actually in running water, they are at least so situated as to receive a constant supply. The finest, most vigorous specimens I have ever collected have been found growing in such a position as to receive constant dripping of fresh water from above, which so far as has been observed does not materially influence the development of fruiting organs as is the case when *Fegatella* is too abundantly supplied.

Several specimens of *Ricciocarpus natans* found growing on soil about a small lake in the foot-hills were brought into the laboratory along with enough of the substratum to prevent any shock resulting from transference. These along with some *Aneura multifida major* were placed in a shallow earthern saucer and exposed to the dry air of the laboratory until in a normal air-dry condition. In this condition they were gradually moistened but no sign of recovery was apparent in any part of the tissue of either plant, and after several days of normal moisture supply they showed no signs of life. It is apparent from this and several other experiments upon hygrophilous forms that they lack the degree of adaptability to changed climatic conditions exhibited by such forms as *Riccia*, *Targionia* or *Porella*.

Aside from several genera of Ricciaceæ and Marchantiaceæ, two species of *Sphærocarpus* and one of *Fossombronia*, *F. longiseta*, represent the thallose Jungermanniaceæ for the region about San Francisco Bay, as well as higher up into the hills and mountains. So far as habit is concerned *Sphærocarpus* is hardly to be compared to *Fossombronia* for the one is an annual while the other remains alive throughout the entire summer. We have here a relatively small representation of leafy liverworts, the most common being *Porella bolanderi*, and *Frullania bolanderi*, while in certain isolated localities one may find *Radula complanata* growing on tree trunks and *Lophozia ventricosa* forming reddish-brown patches on exposed hillsides that receive abundant moisture during the rainy season.

The Anthocerotaceæ are represented by *Anthoceros fusiformis*, *A. pearsoni* and *A. phymatodes* the first two being very common, while the third, though not so widely distributed, is very abundant in certain localities near Stanford University. All three species are found growing luxuriantly in places of severe exposure, though thriving best in slightly shaded localities. At one time it was supposed that *A. fusiformis* and *A. pearsoni* were annuals but they were afterwards shown by Campbell[1] to be perennial in habit. So far as the writer has observed *Anthoceros phymatodes* survives through the agency of its conspicuous tubers. In fact, it seems that virtually all of the thallus dies during the dry season except these structures which are packed with food-materials and serve as very effective water-storage organs. The other two species, while surviving the dry season, resume development with but a comparatively small amount of the old thallus persisting, though both species are highly mucilaginous and especially adapted to extreme conditions as described by Campbell.[2] Careful examination of a large number of recently revived plants failed to demonstrate the presence of sex organs even in their early stages and it is probable that these develop after the new growth of thallus is well under way. With the exception of the two species of *Sphærocarpus* it has been shown that all of our xerophytic forms are perennial, persisting from year to year unless artificially interfered with. Just how extended a period of desiccation they are capable of withstanding has never, so far as I know, been determined. In the autumn of 1903 the writer attempted to germinate spores of *Fossombronia longiseta* that had been collected in 1896. Some of the old plants along with the spores were placed in petri dishes containing distilled water, while others were sown upon moist earth. None of the spores germinated nor did the plants revive. The material was sent me from an eastern herbarium and I do not know the conditions affecting the material after collection. The failure of the plants to revive and of the spores to germinate can throw little light upon the question.

[1] Campbell, 1904: Resistance of Drought by Liverworts. Torreya, Vol. 4, No. 6, p. 84.

[2] Campbell, 1895: 1st ed., Mosses and Ferns, p. 117; 2d ed., 1905, p. 123.

So long as the water of constitution is not disturbed the degree of desiccation seems to make no perceptible change in the reviving powers of all our common xerophytic forms. In the preparation of a previous paper[3] quantitative experiments were made upon *Fossombronia longiseta* to ascertain the actual water content of air-dried plants and to test the vitality of plants and spores that had been for several weeks subjected to the action of such a powerful drying reagent as glacial phosphoric acid. The results obtained showed that the plants of this species in the normal air-dry condition contain an appreciative amount of water which may be removed without the slightest injury to the vitality of the plant, while spores subjected to the same treatment germinate quite as readily as those under perfectly normal conditions. To extend these results the same experiment was carried out with *Targionia hypophylla*, *Fimbriaria californica*, *Riccia glauca*, *Porella bolanderi*, *Cryptomitrium tenerum* and again with *Fossombronia longiseta*. All material was collected early in September before the first rains, was brought into the laboratory where, with a fine stiff brush and other instruments, it was carefully freed from any soil particles that might be clinging to the rhizoids. Then to correct an error that might creep in from the absorption of moisture by the plants from the fingers or breath of the operator the plants were again subjected to normal air-dry conditions a number of days.

Following this the five lots of each plant, were carefully weighed on a chemical balance and then placed at once in the receiving chamber of a desiccator over a quantity of glacial phosphoric acid. Here the material was left from September 21, 1906, until February 9, 1907, when it was again weighed and the respective weight losses recorded. The weights before and after artificial drying are given below:

Fimbriaria.	Normal Air-dry Weight.	Loss of Weight After Four Months Desiccation.
lot 1	0.05 g.	0.0025 g.
lot 2	0.05 g.	0.0007 g.
lot 3	thrown out as defective.	
lot 4	0.072 g.	0.0091 g.
lot 5	0.105 g.	0.0047 g.

[4] Loc. cit.

Targionia.	Normal Air-dry Weight.	Loss of Weight After Two Months Desiccation.
lot 1	0.055 g.	0.0035 g.
lot 2	0.070 g.	0.0150 g.
lot 3	0.052 g.	0.0066 g.
lot 4	0.075 g.	0.0046 g.
lot 5	0.055 g.	0.0017 g.

Cryptomityium.	Normal Air-dry Weight.	Loss of Weight After Four Months Desiccation.
lot 1	0.050 g.	0.00250 g.
lot 2	0.040 g.	0.00310 g.
lot 3	0.040 g.	0.00325 g.
lot 4	0.060 g.	0.00230 g.
lot 5	thrown out as defective.	

Riccia glauca.	Normal Air-dry Weight.	Loss of Weight After Two Months Desiccation.
lot 1	0.030 g.	0.00170 g.
lot 2	0.035 g.	0.00200 g.
lot 3	0.030 g.	0.00260 g.
lot 4	0.030 g.	0.00310 g.
lot 5	0.025 g.	0.00330 g.

Fossombronia.	Normal Air-dry Weight.	Loss of Weight After Four Months Desiccation.
lot 1	0.025 g.	0.0020 g.
lot 2	0.020 g.	0.0020 g.
lot 3	0.040 g.	0.0020 g.
lot 4	0.030 g.	0.0026 g.
lot 5	0.025 g.	0.0021 g.

Porella.	Normal Air-dry Weight.	Loss of Weight After Four Months Desiccation.
lot 1	0.420 g.	0.0215 g.
lot 2	0.320 g.	0.0180 g.
lot 3	0.350 g.	0.0150 g.
lot 4	0.402 g.	0.0170 g.
lot 5	0.402 g.	0.0151 g.

The wide discrepancies existing between the weights of different lots of *Fimbriaria* and of *Targionia* are probably due to the fact that certain lots were largely made up of thick, bulky plants capable of retaining more moisture than the smaller thinner plants composing other lots. Then too, some allowance must be made for small particles of soil clinging to the rhizoids, though care was exercised in the removal of all foreign matter from the plants employed in the experiment. It will be noticed that in the case of *Cryptomitrium*, *Fossombronia* and the others where the plants were of more uniform size the discrepancies are slight.

Of the above forms *Fimbriaria*, *Targionia*, and *Porella* are the most sensitively hygroscopic; at least when moistened they are the first to become turgid and resume activity.

By dividing the total loss in weight due to artificial desiccation by the air-dry weight we obtain in percentages the actual fraction of the air-dry weight that is lost in drying. For example it was found that *Fimbriaria*, lost 6.06 per cent. of its air-dry weight while losses for the other five were: *Targionia*, 12.3 per cent.; *Cryptomitrium*, 5.86 per cent.; *Riccia*, 8.47 per cent.; *Fossombronia*, 8.23 per cent.; *Porella*, 4.57 per cent.

From these figures it will be seen that in the normal air dry state, *Targionia* contains the greatest amount of the water of constitution, while *Fossombronia* and *Riccia* contain about two thirds as much. *Porella*, the only leafy liverwort used in the experiment, had given up all but a very small percentage of its air-dry weight followed closely by *Cryptomitrium*, one of the Marchantiaceæ.

As a rule our summers are characterized by heavy morning fogs which drift in over the mountains from the ocean. These hang over the valley for three or four hours after sunrise greatly reducing the temperature and leaving behind, each morning of their occurrence, considerable precipitation, enough sometimes to result in dripping from eaves. The past summer (1906), however, was unusually free from these fogs, even late in the season when ordinarily they are heaviest and most frequent. Fogless mornings are characterized here by slight or even no precipitation of dew and are forerunners of hot, extremely dry days. During such a season as the one just described it is probable that our liverworts remained in an absolutely dormant condition. It has many times been demonstrated that all our perennial forms on being moistened revive in from half an hour to twelve hours, resuming growth very promptly if the supply of moisture is constant. Campbell[1] has shown in a recent paper that this peculiar adaptation is a property not only of certain of the hepaticæ but belongs also to *Gymnogramme triangularis*, a fern common to this region, which dries up in summer, remaining dormant throughout the season, without the resulting death

[1] Loc. cit., p. 85.

of the leaves as occurs in the majority of ferns. Not infrequently one finds in the field quite fully developed prothallia of this species early in the autumn before the winter rains have actually set in. It is hardly possible for these to have developed from spores that had germinated at any other time than in spring or early summer. In fact, it has been experimentally shown by Professor Peirce[1] that the prothallia of this species may endure prolonged desiccation without evidence of the slightest injury, for on being moistened they very promptly revive and young sporophytes develop normally from these gametophytes of the preceding season. Perennial prothallia developing tubers have been reported by Goebel[2] for the allied fern *Anogramme chærophylla*, common in southern Europe. Such structures belong to some of our liverworts but have not yet been shown to occur in the prothallia of any of our ferns. Aside from the common fern *Gymnogramme*, other pteridophytes native to California, *e. g.*, *Selaginella biglovеii* and *S. lepidophylla*, the latter the well known "resurrection plant," are reported by Campbell in the paper above referred to as remaining in a dry and dormant condition throughout the greater portion of the year, resuming active growth during the rainy season, at first absorbing considerable water through their leaves as do the leaves of *Gymnogramme*.

To test the vitality of those liverworts that had been naturally and then artificially desiccated, a certain number of plants from each lot were placed on moist earth and others in Knop's nutrient solution. In both cases the plants revived promptly and are now growing vigorously, producing fruiting organs in great number. A surprisingly small portion of the thallus of *Targionia*, *Riccia*, *Fimbriaria* and *Porella* was actually dead; though in *Fossombronia*, *Cryptomitrium* and *Anthoceros phymatodes* only the *apical* end including a small portion of the thallus appeared to be alive. Within five hours revived plants from each species were fixed with chromic one per cent. fixing solution and run up through the alcohols and bergamot oil to

[2] Campbell, 1904: Resistance of Drought by Liverworts. Torreya, Vol. 4, No. 6, p. 85.

[3] Goebel, 1898: Organography of Plants. Part II, Vol. 1, p. 426.

paraffin. The same method of fixation, dehydration, clearing and infiltration were employed here as detailed in a previous paper on the development of *Fossombronia longiseta*, barring slight modifications. It was found that the best results could be obtained by following the method of dehydration described by Chamberlain.[1] The use of diffusion shells and the "constant drip" process have proved somewhat unreliable, not always giving satisfactory results for such tissues as prevail among the more delicate hepaticæ. Instead of transferring from absolute alcohol directly to a 50 per cent. solution of bergamot oil and then to pure bergamot, a more gradual transfer was employed and a similar plan was followed in running up through the paraffins.

In the autumn of 1903 the writer revived some material consisting of *Fossombronia* and *Fimbriaria* and after a few hours placed them in a fixing solution. On studying sections of each it was observed that in the case of *Fossombronia*, not only was a considerable portion of the thallus alive but it was found that there were nearly mature antheridia and archegonia. Sections of *Fimbriaria* through the median sulcus proved the presence of well-advanced antheridia. This suggested the possibility of a similar early development of sex organs in other forms than the two mentioned above. A number of slides of *Riccia*, *Targionia*, and *Cryptomitrium* as well as of *Fossombronia* and *Fimbriaria* were prepared and in each instance, at least, antheridia have been found. Only in *Fossombronia* and *Porella* have we found archegonia. In *Fimbriaria* and *Cryptomitrium* these organs occur on special receptacles none of which showed any sign of development. In *Riccia* and *Crytomitrium* only early stages of the antheridium were present which was rather contrary to anticipation. A large number of plants of each genus were sectioned and the great majority of them were sterile. Quite early in the rainy season we have been in the custom of collecting quantities of *Riccia* in which no difficulty is experienced in obtaining virtually all stages in the development of the sex organs. For this reason the writer feels that a more exhaustive examination of this species ought to reveal

[1] Chamberlain, 1905: Methods in Plant Histology, p. 22.

fruiting organs quite as advanced as those of *Targionia* or *Fimbriaria*. As for *Cryptomitrium*, it is not surprising to find so few antheridia, for even as late as the first week of February sections of *Cryptomitrium* revealed the presence of mature antheridia while in the case of *Fimbriaria* they may be found almost ripe before the beginning of the wet season. Abrams[1] in his studies of *Cryptomitrium* found that the antheridia matured earlier than the archegonia and informs me that he found fairly well advanced stages of the antheridium as early as November. In *Porella* not only all stages in the development of sex organs are to be found but well-developed sporophytes as well. This accounts for the very sudden appearance of mature sporophytes shortly following a few days of wet weather. Growing as it ordinarily does on the exposed surfaces of rocks and tree trunks, it is liable to severe exposure and may be left quite dry in a few hours of wind or sunshine. This fact may have some influence upon its reproductive habits and, in a measure, account for the advanced sporogonia found in revived material. At any rate *Porella* is scarcely to be compared with such thallose forms as *Riccia* or *Targionia*. The differences in habit and structure might well beget differences relative to the appearance and time of development of the sex organs.

In another part of this paper the writer mentions having found *Fossombronia longiseta* so situated out of doors as to receive a constant supply of moisture from a leak in a water pipe. Many of these plants were examined on November 1 very near the close of the dry season and were found bearing embryos in various stages of development and in some instances well advanced sporogonia as shown in Fig. 1. By the first of January the spores and elaters had matured and some capsules had already dehisced.

Not having observed these plants at the time of fertilization it cannot be definitely said just when the antheridia and archegonia matured. However, the approximate date may be ascertained by observing the rate of development of the sporogonium and of the ripening of the spores. In 1904 the first rain of

[1] Abrams, 1899: Structure and Development of *Cryptomitrium tenerum*. Botanical Gazette, Vol. 28, pp. 110–121.

any consequence fell on the twenty-first of August, but liverworts barely revived by it were overtaken by a period of dry, hot weather which lasted till September 23, when the rainy season began. It might justly be assumed that the resumption of growth, as regards *Fossombronia* at least, began at this time, More than the usual amount of rain was recorded for October and November and growing conditions were very favorable. Material collected in early February showed sporongia in which the spore-mother cells were preparing to pass into the tetraspore condition, and six weeks later material was collected bearing ripe spores and elaters. From this it will be seen that, having a favorable rainy season, the time required to produce ripe spores is approximately six months after the first rains of any consequence. Judging from the advanced development in which one finds antheridia and archegonia at the close of the summer season, fertilization must occur within a few weeks after resumption of growth is well established.

With regard to the plants that had grown unchecked throughout the summer, it must be noted that temperature and humidity conditions are widely different from those prevailing in winter. Though the plants were well irrigated, the atmospheric conditions were such as to influence to some extent the growth and behavior of the thallus and fruiting organs. Assuming, however, the same rate of development of the sporogonia for these plants as for those grown in winter, fertilization must have occurred during July and later. Growing in a paraffin-coated glass dish containing Knop's nutrient solution, the writer has under observation several plants of *Fossombronia*, placed there a month ago in air-dry condition as nearly free from soil particles as they could be rendered by the use of a fine, stiff brush. Antheridia and archegonia then in the initial stages of development are now about as far advanced as were the oldest of those observed as soon as the plants had revived — in the course of twelve hours. These oldest ones are now maturing. It will be shown elsewhere, however, that Knop's solution affords a very active stimulus to development of the thallus and fruiting organs, probably shortening the time of their normal development considerably.

At any rate, it is evident that under conditions prevailing in this part of California a second crop of antheridia appears before the close of a single growing season, and in the case of *Fossombronia* at least, when the supply of moisture is maintained, fertilization and ultimate development of the sporophyte proceed regularly, the spores ripening three or four months earlier than those of the winter crop. It is probable that the second growth of antheridia and archegonia begin to develop at least a month before the close of the rainy season, and this fact is of particular importance to the plant as it insures the maturity of the sporophytic generation well within the rainy season.

Certain of our liverworts show a tendency to develop tubers or other structures enabling them to resist drought. For example, in the southern part of the State, *Geothallus tuberosus* is a form bearing well-marked tubers, while in our own locality *Fossombronia* develops tuber-like thickenings of the stem, and well-defined tubers are to be found in the case of *Anthoceros phymatodes*. All the plants employed in this study showed the presence of scales or hairs which in some instances secrete mucilage; these along with the mucilage cells within the thallus are structures undoubtedly serving as water-storage organs.

According to Howe,[1] aside from *Anthoceros phymatodes*, tuberous structures are not uncommon among plants of *A. pearsoni*, both species growing ordinarily in exposed places. In other parts of the world, hepaticæ bearing tubers have been found. For example Goebel[2] describes two species of *Anthoceros*, *A. argentinus* and *A. dichotomus* and a species of *Fossombronia*, *F. tuberifera*, common in certain parts of Chili. He regards the organ in the last named species a true tuber not unlike the same structure developed by our *Anthoceros phymatodes*, but considers the tubers of *A. argentinus* and *A. dichotomus* as transformed branches of the thallus, the ends of which, have become swollen and filled with reserve food products, very similar to what prevails in our common species *A. pearsoni*. Goebel[3] also reports certain species of *Riccia* as developing tubers,

[1] Loc. cit., p. 184.
[2] Loc. cit., p. 293.
[3] Loc. cit., p. 70.

notably an Italian form in which he observed entire portions of the thallus developed into tuber-like structures. Stephani[1] has also described similar structures for *Riccia bulbifera*. None of our Californian Riccias has yet been described as bearing tubers or tuber-like growths, though *Riccia trichocarpa*, mentioned elsewhere in this paper, is well adapted to withstand severe exposure, the thallus being densely clothed with tawny setæ .3 to .65 mm. long, with those toward the apex in as many as eight to twelve series, affording a very effective protection against excessive drying. Though these tuber-like structures wherever they have been examined are always found to contain large quantities of reserve food, there is little doubt that they are instrumental as water-storage organs.

STUDIES IN THE GERMINATION OF SPORES.

In a previous paper the writer has described and figured the germination of the spores of *Fossombronia longiseta*. The results of experiments then carried out showed the early development of this species to agree closely with certain forms, notably *Sphærocarpus californicus*, investigated by Campbell.[2]

Most of the hepaticæ of this region produce spores characterized by a heavy, more or less sculptured exosporium which Leitgeb[3] found to consist of two parts or layers of which the inner belongs to the spore itself and is the exine, while the perinium or outer layer is formed later and consists of parts of the sporocyte. Leitgeb suggests the probability of the perinium as a protection against drought but in the light of more recent studies of certain forms producing thick-walled spores, *e. g.*, *Corsinia*, *Preissia*, *Anthoceros* and *Sphærocarpus*, it may be held that the perinium can certainly have nothimg to do with a resting period, for spores of these forms germinate readily within a few days after being sown. As to the thick exosporium furnishing a protection against excessive dryness there can be little doubt, although such forms as *Corsinia* and the aquatic Ric-

[1] Stephani, 1898: Species Hepaticarum. Bull. de l'Herb. Boiss., Vol. VI, p. 333.

[2] Campbell, 1896: Notes on Sphærocarpus. Erythea, Vol. IV, No. 5, Pl. 2.

[3] Leitgeb, 1884, Ueber Bau und Entwicklung der Sporenhaute, Graz.

cieæ produce spores of similar character. Goebel,[1] however, suggests that localities favorable to *Corsinia* may not always be moist, and in the case of the aquatic Riccieæ the perinium very effectively serves as a protection against invasion of the spore by fungal hyphæ.

Although the great majority of our xerophilous liverworts produce thick-walled spores there are such exceptions as *Porella bolanderi* and *Radula complanata* whose spores are protected by a thin, frail exosporium and are incapable of withstanding prolonged drying. These liverworts grow on tree trunks or exposed surfaces of rocks where they are subject to sudden drying out and we should naturally expect their spores to be better protected.

The character of the spore-wall, however, might suggest the possibility of these forms being naturally more hygrophilous in character, while their adaptation to a dry habitat is merely one phase of the extreme adaptability common to both forms.

In order to get at the relationship of the exospore to the spore a study of the life conditions of the several species is necessary and some light will be thrown upon the subject when we ascertain at what time, in nature, the spores of a large variety of forms germinate. With a large number of forms it has been repeatedly shown that spores sown on soil under conditions as nearly normal as possible germinate only after a long period of rest.

In June, 1904, the writer sowed on sterilized earth, a quantity of spores of *Fossombronia* that had been gathered a few days before sowing. The culture was made in an earthen saucer and placed within another close-fitting earthen saucer, in which was maintained a supply of water so that the dirt was kept constantly moist from below. The culture was placed on a table near a window where it could receive a nearly normal supply of light. During the latter part of August a few spores had germinated but not until October did they germinate in any great number. The spore of *Fossombronia*, while perhaps not as well protected as that of *Fimbriaria* or *Targionia*, nevertheless is furnished with a resistant exosporium and is well adapted to

[1] Loc. cit., 107.

the xerophilous conditions under which the plant grows. The spores are positively known to maintain their vitality for at least two years but for how much longer, cannot be said. The thin-walled spores of such forms as *Aneura*, *Fegatella*, *Radula* and *Porella* withstand but little drying, losing all germinating power within a few months at most, which fact would seem to be evidence in favor of the statement that the thick exosporium is a protective structure.

Spores of *Anthoceros fusiformis* one year and eight months old, when sown on sterilized soil germinated copiously within two weeks. Here we have an instance of spores that are known not to require a resting period before germinating, yet, unlike *Aneura*, the spores of *Anthoceros* are known to endure at least two years desiccation. Spores of *Fimbriaria californica* and *Targionia hypophylla* two years of age were sown on sterilized earth and kept well watered. These germinated within twelve and fifty-six days respectively. This wide discrepancy is difficult to account for. The soil was kept as free from fungi as possible: however, only a few of the *Targionia* spores germinated and this fact may have been due to reduced vitality from prolonged drying. Further experiments with spores of this genus are necessary ; in fact, germination experiments conducted out of doors are the ones of greatest value. Such a series of culture experiments was started more than a year ago in the Stanford Arboretum but before results of any value were obtained the cultures were accidentally destroyed by a workman. It was then too late in the season to start another series, it being very near the close of the rainy season. A fresh collection of spores was made from the 1906 crop and several artificial cultures were started indoors, using both sterilized and non-sterilized earth and Knop's nutrient solution. The spores used in the various cultures were of the following forms : *Riccia glauca*, *Fimbriaria violacea*, *F. californica*, *Targionia hypophylla*, *Fossombronia longiseta*, *Cryptomitrium tenerum* and *Aneura multifida major*.

No spores of the first mentioned plant have yet germinated, though sown six months ago after a resting period of five months. Spores of both species of *Fimbriaria* failed to ger-

minate, though sown at the same time as those in the case of *Riccia*. A very few of the spores of either *Fossombronia* or *Cryptomitrium* germinated. Though he did not duplicate in every case the cultures of the writer, Professor Peirce had a similar experience with all spores of the same (1906) crop. This apparent loss of vitality is difficult to account for, unless due to the unusual character of the weather during the spring months. Ordinarily our last rains occur in April, by which time the spores of the above-named hepaticæ have either been discharged or are maturing. An examination of the daily record of precipitation from April 1 to the close of the season may be instructive. Up to April 1 from January 1 the total rainfall amounted to 10.04 inches. From April 1 to June 15 the record was as follows:

Date.		Amount of Precipitation.	Date.		Amount of Precipitation.
April	1	0.06	May	14	0.02
"	10	0.01	"	25	0.34
"	23	0.11	"	26	0.04
"	24	0.03	"	27	0.51
"	27	0.29	"	28	0.11
"	29	trace	"	28	0.11
May	10	0.01	June	3	0.02
"	11	0.02	"	5	0.73
			"	15	0.24
			"	26	trace

From the above record it may readily be seen that from April 1 to April 23 the total precipitation amounted to but .07 of an inch — scarcely more than a trace. Between the twenty-third and twenty-seventh enough rain fell to revive the already dried mosses and liverworts to a condition of perfectly normal turgidity and they remained so at least a week after the last rainfall. Now during April the spores of a number of our liverworts are only beginning to mature, and it is barely possible that the prolonged dry period during that month interfered with the proper ripening of the spores. On the other hand, spores of certain forms, for example, *Fossombronia*, which had fully matured during March but were not gathered till after the rainy season, failed to germinate; and this suggests the possibility of the vitality of the spores having been impaired by successive

rains and rapid desiccation following one upon the other. Whatever be the cause of impairment the fact remains that almost no spores germinated even under most painstaking precautions as to culture conditions. To get at the root of the matter, however, a series of experiments is necessary in which the student should duplicate as nearly as possible the conditions prevailing from April 1 to June 15 of 1906.

It is an unsettled question as to whether the spores of many liverworts actually require a prolonged resting-period, and there is still need of investigation along this line. We are familiar with the fact that the spores of most xerophilous forms do lie dormant several weeks before germinating but it has not been shown in very many instances that this resting-period is intimately connected with the preparation of the spore for germination. The writer has demonstrated beyond doubt that the spores of *Fossombronia longiseta*, under certain conditions, do not require a resting-period. Cultures were prepared using different media with a view to testing, if possible, the relative influences of the various media upon germination. One lot of spores collected on January 10, 1906, were sown January 12 on pulverized earth that had been thoroughly steam-sterilized and well watered with distilled water. Care was exercised to select soil on which *Fossombronia* grows vigorously out of doors; this was placed in a crystallization dish and covered with one half of a petri dish to keep the culture as free from dust as possible. A number of crystallization dishes were thoroughly cleaned and then covered on the inside with a thin coating of paraffin to prevent the water used from taking up any impurities that might exist in the glass. In three of these, Knop's nutrient solution of the following proportions was placed:

H_2O	500 c.c.
Calcium nitrate	2.00 grams.
Magnesium sulphate	0.5 "
Potassium nitrate	0.5 "
Potassium phosphate	0.5 "

Other solutions of varying strength were employed as follows:

H_2O	500 c.c.
Calcium nitrate	2.00 grams.
Magnesium sulphate	0.5 "
Potassium nitrate	0.5 "
Potassium phosphate omitted.	

Other stock solutions were made up in which the amounts of the various salts used in a normal solution were reduced in quantity one fourth and one eighth, though the quantity of distilled water was kept the same, 500 c.c., in all the solutions. It will be noticed that from one solution the acid salt, potassium phosphate, was left out with a view to testing its value as a stimulus to germination. Spores of the same lot were sown in the various solutions the same day they were collected; the culture dishes were carefully covered and placed on a north window-sill where a uniform amount of diffused light fell upon each culture. In another dish that had been paraffin-coated, water that had been twice distilled was placed and on the same date spores of the same lot were sown. These germinated in considerable numbers within twelve days. Some of the water used in this culture was taken from the supply bottle and evaporated on a slide. On examination, a precipitate of organic particles was found, showing that certain compounds had passed over with the water in distilling, and it was thought probable that these acted as a stimulus, for the spores in this culture germinated before those in the culture containing none of the acid salt. In fact these latter seemed to show no indications of any preparation for germination, such as change in color of exosporium from very dark brown to a lighter more translucent shade. The distilled water used in the preparation of the various culture solutions was from the same stock bottle as that used in the distilled-water culture. Why spores collected at the same time and sown at the same time on these two media should have germinated earlier in distilled water containing organic impurities than in a culture solution void of one of its constituent salts is difficult to account for unless in the latter we have a physiologically unbalanced solution which has been shown by Loeb[1] to exercise a certain toxic effect upon certain marine and fresh-water animals and by Osterhout[2] in his experiments upon algæ. This, however, needs far more extensive investigation than has yet been given to it.

[1] Loeb, 1905: Pflüger's Archiv, 107: 252.

[2] Osterhout, 1906: On the Importance of Physiologically Balanced Solutions for Plants. University of California Publications. Botany, Vol. 2, No. 10, pp. 229–230.

The spores in the unbalanced culture solution germinated within six days after those in the normal Knop's solution, which would indicate the existence of some stimulating factor in that solution. Another lot of distilled water was then prepared by allowing the first fourth or fifth part to distil as waste water, thus getting rid of the larger part of the volatile substances. Only the middle three fifths was saved and this was redistilled in the same manner. The best portion of this was again distilled and only the middle portion of the product was collected; in this way water of a high degree of purity was obtained. This was used in the preparation of another distilled water culture and spores of *Fossombronia* sown eighteen days ago show no evidence of germinating. Spores sown on Knop's solution, normal, germinated within fourteen days producing vigorous germ tubes with rather more than the usual amount of chlorophyll. These young plants have grown more rapidly and vigorously than those in distilled water that germinated approximately at the same time. Spores sown in the normal solution germinated two days later than those in either of the reduced solutions but in the former there was a far greater number of young plants two days after the first observation of germination.

Spores of *Fimbriaria californica* collected in March, 1905, were sown in Knop's solution (normal) and germinated promptly within twelve days. Young plants in this culture that are now sixteen to twenty days old are characterized by the unusual length of the germ-tube, in some instances measuring more than one and a half millimeters from the basal end to the first cell cut off at the distal end of the germ-tube. In some instances two germ-tubes were seen to emerge from a single spore as figured by Campbell[1] for *Targionia hypophylla*. Spores of the same species were sown in an earthen saucer filled more than half-full of finely pulverized non-sterilized soil. These germinated promptly and in great numbers. Both cultures were so placed as to receive light from a north window: the Knop's solution culture received somewhat the greater amount of light being in a glass dish, coated part way up with paraffin. None of the young plants of the soil culture produced germ-

[1] Loc. cit., 1st ed., p. 65; 2d ed., p. 66.

tubes of more than usual length while some showed the formation of cross-walls very early. The young plants in the nutrient solution have grown rapidly, producing in some instances as advanced a development as is seen in Fig. 21, within eighteen days. A very few young plants in which there was only a slight germ-tube development had already begun to assume the dorsi-ventral form common to this genus. Desiccated plants of *Fimbriaria*, *Riccia* and *Fossombronia* when placed in Knop's solution revived quickly producing within a few days a greatly increased number of rhizoids and in the case of *Fossombronia*, many more than the usual number of antheridia, closely covering the dorsal surface of the young growing shoot. From these and other results it seems highly probable that Knop's nutrient solution exerts a stimulative influence upon the development of the thallus, rhizoids, and reproductive organs as well as the germination of the spores. Further investigation, however, with solutions of varying strength and composition is needed in order definitely to determine the nature and chemical composition of the salt or combination of salts that act as a stimulus to growth.

According to Goebel,[1] germination in the Marchantieæ when compared with other thallose liverworts presents certain characteristic differences. He separates the product of germination of a spore of *Preissia* for example, into three structures, *i. e.*, the germ-tube, the "pro-embryo" and the young plant. He finds that the "pro-embryo" is positively heliotropic and develops at its end a flattened cell mass which he terms the germ disk. This forms at right angles to the direction of the light-rays and the young plant arises from one quadrant of the germ disk as shown in his Fig. 95.

On the same page he states that a similar germination is common in the Riccieæ contrary to the description and figures of Campbell[2] who studied the germination of *Riccia trichocarpa* and a number of other forms. He refers to Campbell's Fig. 9, p. 38, as failing to bear out his statement that the axis of growth in the young plant is continuous with that of the germ-tube.

[1] Loc. cit., p. 111.

[2] Loc. cit., 1st ed., p. 39; 2d ed., p. 38.

An examination of the figure referred to, however, will show that although an angle was present in the young gametophyte it developed in the germ-tube itself, the upper portion of which lies in the direct axis of growth of the young plant, no such structure as a pro-embryo being present. In his study of *Fimbriaria* and *Targionia* his figures show an early development of the young plant not unlike that in the case of *Riccia trichocarpa*. The writer's investigations relative to the germination of both *Fossombronia* and *Fimbriaria* agree in detail with those of Campbell in the forms studied by him (Figs. 22 and 23).

MacDougal[1] has shown that, given the necessary intensity of light, its direction will vitally influence the form and habit of a living plant as well as the disposition of various organs incident to its development. This being true, we might, *a priori*, assume that Goebel's *Preissia* and *Riccia* spores on germinating take on the angular form shown in his Fig. 95 because of certain external factors and, most of all, light, instead of trying to account for it on grounds of heredity or of certain inherent factors. In fact, Peirce,[2] Rosenvinge,[3] and others have experimentally shown that in the case of certain algæ, germinating spores are profoundly influenced by the direction and intensity of light. Eliminating so far as possible all other external factors it was found that the young plants always grew towards the light, while the rhizoid or the holdfast was negatively heliotropic. Peirce has demonstrated that in addition to this, light exerts a directive influence upon the formation of cross-walls in sporelings of algæ, liverworts and certain ferns, these walls always forming at right angles to the direction of the light rays.[4]

With ferns and liverworts he has further shown that when the long axis of the germ-tube lies in the direction of the light rays, the continued growth of the young plant will be in the same direction. More than this, he has demonstrated that when the germ-tube ruptures the spore wall on the side away from the

[1] MacDougal, 1903: The Influence of Light and Darkness upon Growth and Development. Memoirs of the New York Botanical Garden, Vol. II.

[2] Loc. cit., p. 338.

[3] Rosenvinge, 1889: Influence d'agents exterieurs sur l'organisation polaire et dorsiventral des plantes. Revue Generale de Botanique, 1.

[4] Peirce, 1906: Studies of Irritability in Plants, p. 453.

light there is a strong tendency to turn toward the light, and if this is not accomplished by the germ-tube, the further development of the young plant will at once or gradually assume the direction of positive heliotropism.

DURATION OF VITALITY OF CERTAIN GREEN OR THIN-WALLED SPORES.

A failure to germinate the spores of *Aneura multifida major* after they had been allowed to become several weeks old, induced the writer to prepare a series of cultures to test the life duration of the spores of *Aneura*, *Radula*, *Porella* and *Fegatella*.

The first spore cultures of all these forms were started on the same day of collection in order to determine whether the spores were ready for germination under normal conditions. Practically all spores germinated in cultures consisting of sterilized earth, Knop's solution and distilled water. I next allowed spores of *Fegatella* to dry for three days, by placing them on a sheet of paper on the laboratory table. These were then sown in the various culture media. Many of the spores had already germinated within the capsule but these along with all spores still ungerminated failed to show the slightest sign of any recovery after days of constant moisture and favorable light. Later, Professor Peirce demonstrated that one hour's drying under normal conditions was sufficient to kill all spores, and further experiments with the spores of this species were regarded unnecessary. The spore wall in the case of *Fegatella* is very thin, allowing rapid loss of the water of constitution, resulting in a general contraction of spore contents and collapse of the spore wall.

On January 12 cultures were started, sowing spores of *Radula complanata* collected on the ninth of the same month. Practically all of these germinated within a few days, so another trial was not made till the twenty-eighth of January. Approximately ten per cent. of the spores of this sowing failed to recover, the cytoplasm and other spore contents having contracted towards the center forming a brownish mass.

On February 11 a third culture was started, the spores now

having dried thirty-two days. Between 20 and 25 per cent. of these spores failed to recover. The last lot of spores were sown on March 3 — fifty-two days after collecting. Approximately forty per cent. of these were desiccated beyond recovery. Time does not permit at the present further testing of the spores of this species but judging from the results of the above cultures very few if any spores could endure longer than a few months at the most.

Porella bolanderi was then experimented with, cultures being started at various intervals using the same media as in preceding cultures. The results obtained were not greatly different from those in the case of *Radula* though in my last culture which was made on March 5 not more than half the spores have recovered and fewer still have germinated after eleven days. The spores in this culture had been allowed to dry thirty-eight days, or fourteen days less than was allowed spores used in the last *Radula* culture. Making due allowance for the possibility of toxic effects due to the activity of bacteria or fungi that might have been in the various cultures it is highly improbable that the failure of constantly increasing numbers of spores to revive could have been entirely due to any such cause and we are justified in the conclusion that the spores of these forms and probably some others not yet investigated are incapable of enduring more than a comparatively slight amount of desiccation.

Summary.

1. Plants of *Fossombronia longiseta*, as it occurs here, are seldom free from infection by a fungus, which, in its relation to the host, acts as a true parasite. In early stages the fungus is confined to the rhizoids and compact tissue of the stem, ultimately extending throughout all vegetative tissues of the gametophyte. Large, black sclerotia develop within the cells of the leaves and peripheral cells of the stem, resulting in the death of the host.

No instance of infection of sex organs or sporogonium has been observed.

2. *Fimbriaria californica* has been found to be infected by a fungus, the hyphæ being confined to the outermost three or

four layers of ventral cells and to both sorts of rhizoids. Its relation to the host is apparently symbiotic.

3. A fungus, evidently epiphytic in habit, was found associated with *Aneura multifida major*. Its relation to the host is that of a parasite.

4. Fungi were also found associated with *Anthoceros pearsoni* and *Porella bolanderi*. In both cases the fungus is epiphytic, sending hausteria-like branches into the tissues of the host.

5. Fertilization in *Fegatella conica* in California occurs in early spring. At the close of the dry season the spores are in the tetrad stage. With the commencement of the rainy season, the growth of the female receptacle is resumed and the spores mature early in January.

6. When hygrophilous forms such as *Aneura pinguis* or *Fegatella conica* are transferred to a dry habitat and allowed to become desiccated they are unable to recover.

7. All of our xerophilous forms such as *Fimbriaria californica*, *Targionia hypoyhylla*, several species of *Riccia*, *Fossombronia*, and others resume growth promptly after months of extreme drought. When artificially desiccated over glacial phosphoric acid until further loss of weight is imperceptible, on being moistened they revive quite as promptly as when normally air-dried.

8. Artificial drying does not impair germination of spores of xerophilous forms.

9. In *Porella bolanderi* material examined at the close of the dry season exhibits holdover antheridia and archegonia and well-advanced sporogonia. Other xerophilous forms of perennial habit show sex organs in various stages of development.

10. *Fossombronia* growing in a habitat of constant moisture matured spores in March and December to February.

11. Mucilage secreting structures are common to many forms and especially to our species of *Anthoceros*. *A. pearsoni* and *A. phymatodes* are rendered resistant to drought by the presence of tubers. Similar structures, though less developed, are common to *Fossombronia longiseta* in dry regions.

12. Spores of xerophilous forms are known to retain their vitality at least two years and probably longer. In nature they pass through a resting period of several weeks.

13. Spores of *Fossombronia longiseta* sown in Knop's solution and in impure distilled water germinated in the former within fourteen days and in the latter within twelve days after removal from the capsule. Spores sown on sterilized soil show no evidence of germination after sixty-two days.

14. The spores of *Fegatella conica*, *Aneura pinguis*, *Porella bolanderi* and *Radula complanata* were found incapable of withstanding prolonged desiccation. Germination occurs normally within the capsule or very soon after dispersal.

BIBLIOGRAPHY.

Abrams.

1899 Structure and Development of *Cryptomitrium tenerum* (Hook.) Aust. Botanical Gazette, Vol. 38.

Bruchmann.

1904 Ueber das Prothallium und die Keimpflanze von *Ophioglossum vulgatum* L. Botanische Zeitung, Heft XII, Tafel VIII.

1906 Ueber das Prothallium und Sporenpflanze von *Botrychium lunaria* Sw. Flora oder Allgemeine Botanische Zeitung. Band 96, Heft 1.

Campbell.

1896 The Development of *Geothallus tuberosus* Camp. Annals of Botany, Vol. IX.

1896 Notes on *Sphærocarpus*. Erythea, Vol. IV, No. 5, Pl. 2.

1895 Mosses and Ferns, 1st ed. 1905, 2d ed.

1904 Resistance of Drought by Liverworts. Torreya, Vol. 4, No. 6.

1907 Studies on the Ophioglossaceæ. Annales du Jardin Botanique, Vol. XXI.

Cavers.

1903 On Saprophytism and Mycorrhiza in Hepaticæ. The New Phytologist, Vol. II, No. 2.

1904 On the Structure and Biology of *Fegatella conica* Corda. Annals of Botany, Vol. XVIII, No. LXIX, Plate VI.

1904 Contributions to the Biology of the Hepaticæ. Part 1, *Targionia*, *Reboulia*, *Preissia*, *Monoclea*.

Chamberlain.

1905 Methods in Plant Histology.

Czapek.

1889 Zur Chemie der Zell-membranen bei den Laub und Lebermoosen. Flora, Band 86.

Goebel.

1898 Organography of Plants, Part II, Vol. 1.

Golenkin.

1902 Die Mychorrhiza ähnlichen Bildungen der Marchantiaceen, Flora, Band 90.

Howe.

1898 The Anthocerotaceæ of North America. Bulletin of the Torrey Botanical Club, Vol. 25, No. 1.

1899 The Hepaticæ and Anthocerotes of California. Memoirs of the Torrey Botanical Club, Vol. VII.

Humphrey.

1906 The Development of *Fossombronia longiseta* Aust. Annals of Botany, Vol. XX, No. LXXVII.

Jeffrey.

1898 The Gametophyte of *Botrychium virginianum*. University of Toronto Studies, No. 1.

Kny and Bottger.

1879 Ueber eigenthümliche Durchwachsungen an den Wurzelharren zweier Marchantiaceen. Verhandl. d. bot. Vereins d. Prov. Brandenburg.

Leitgeb.

Untersuchungen über die Lebermoose, Heft 2, Tafel 3.

1884 Ueber Bau und Entwicklung der Sporenhäute. Graz.

Loeb.

1905 Pflüger's Archiv, 107.

MacDougal.

1908 The Influence of Light and Darkness upon Growth and Development. Memoirs of the New York Botanical Garden, Vol. II.

Osterhout.

1906 On the Importance of Physiologically Balanced Solutions for Plants. University of California Publications. Botany, Vol. 2, No. 10.

Peirce.

1906 Studies of Irritability in Plants. Annals of Botany, Vol. XX, No. LXX.

Peirce and Randolph.

1905 Studies of Irritability in Algæ. Botanical Gazette, No. 40.

Rosenvinge.

1889 Influence d'agents exterieurs sur l'organisation polaire et dorsiventrale des plantes. Revue generale de Botanique. 1.

Stephani.

1898 Species Hepaticarum. Bulletin de l'Herbier Boissier, Vol. VI.

EXPLANATION OF FIGURES IN PLATES I AND II.

FIG. 1. Diagram of a young Sporogonium of ***Fossombronia longiseta*** collected November 1, 1906, near close of the dry season. Found in a locality of constant moisture.

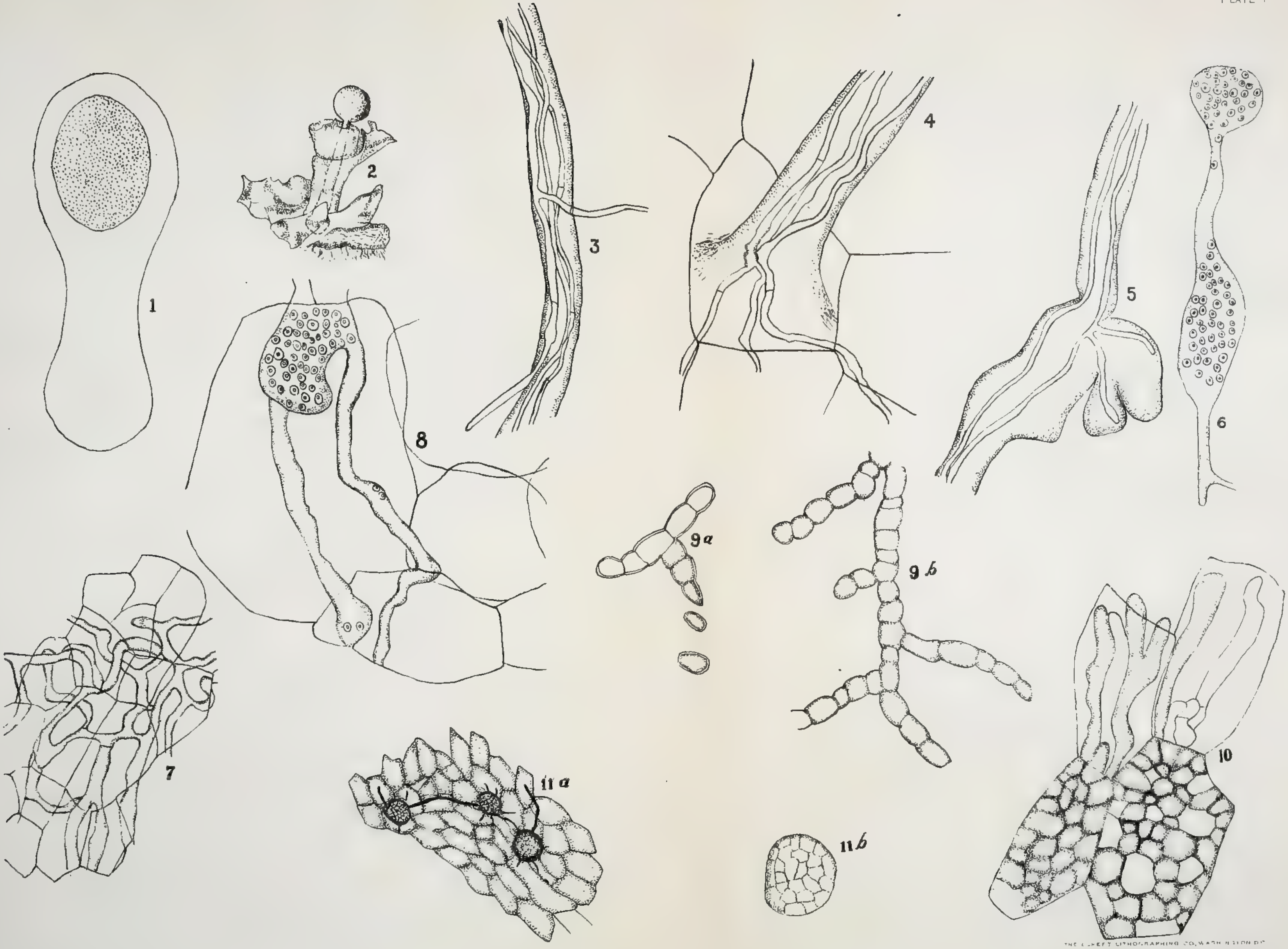
2. Mature plant of ***Fossombronia longiseta***. Had grown all summer. Collected January 6, drawn January 7.
3. Living rhizoid of ***F. longiseta*** perforated by hyphæ. × 560.
4. Hyphæ passing from cells of thallus into rhizoid. × 560.
5. Abnormal rhizoid of ***F. longiseta*** containing hyphæ. × 560.
6. Thin-walled vesicle of the fungus associated with ***F. longiseta***, within stem tissue of the host. × 560.
7. Hypha complex within cells of the host, ***F. longiseta***. × 560.
8. Vesicle and hyphæ apparently communicating with the exterior. × 560.
9. (*a*) and (*b*). Formation of conidia by abstriction of hypha of the fungus infecting ***F. longiseta***. × 560.
10. Development of sclerotia in cells of a leaf of ***F. longiseta***. × 560.
11. Perithecia-like structures developing from hyphæ on the surface of ***Aneura multifida major***.
12. Uninfected cells of ***Aneura multifida major*** showing oil-bodies, ***o. b.***, and chromatophores. × 335.
13. Infected cells of ***Aneura multifida major***. Note the absence of oil-bodies and small number of chromatophores. × 335.
14. Three cells of ***Aneura multifida major*** showing knots of hyphæ. Chlorophyll and other cell contents wanting; cells, dead. × 335.
15. Infection of a young plant of ***Aneura multifida major*** that has developed from a gemma. × 560.

16*a*. Branching rhizoids of ***Aneura multifida major***. × 80.

16*b*. Branching rhizoids of ***Lepidozia attenuata***. × 80.

17. Longitudinal section of young sporogonium of ***Porella bolanderi*** near close of the dry-season. × about 50.
18. Portion of an infected leaf of ***Porella bolanderi*** showing chlamydospore (*c*). × 560.
19. Developing sclerotia within leaf cells of ***Porella***. × 560.
20. Infection of tissue of ***Anthoceros pearsoni***, showing sclerotium. × 560.
21. Portion of plant of ***Fegatella conica*** showing receptacle. Collected December 28, 1906; drawn January 2, 1907. Ad. nat.
22. Apical end of a young ***Fimbriaria*** plant. × 560.
23. Young plant of ***Fossombronia longiseta***. × 560.

All drawings except Fig. 1, 16 and 23 were made with the aid of an Abbé camera lucida.



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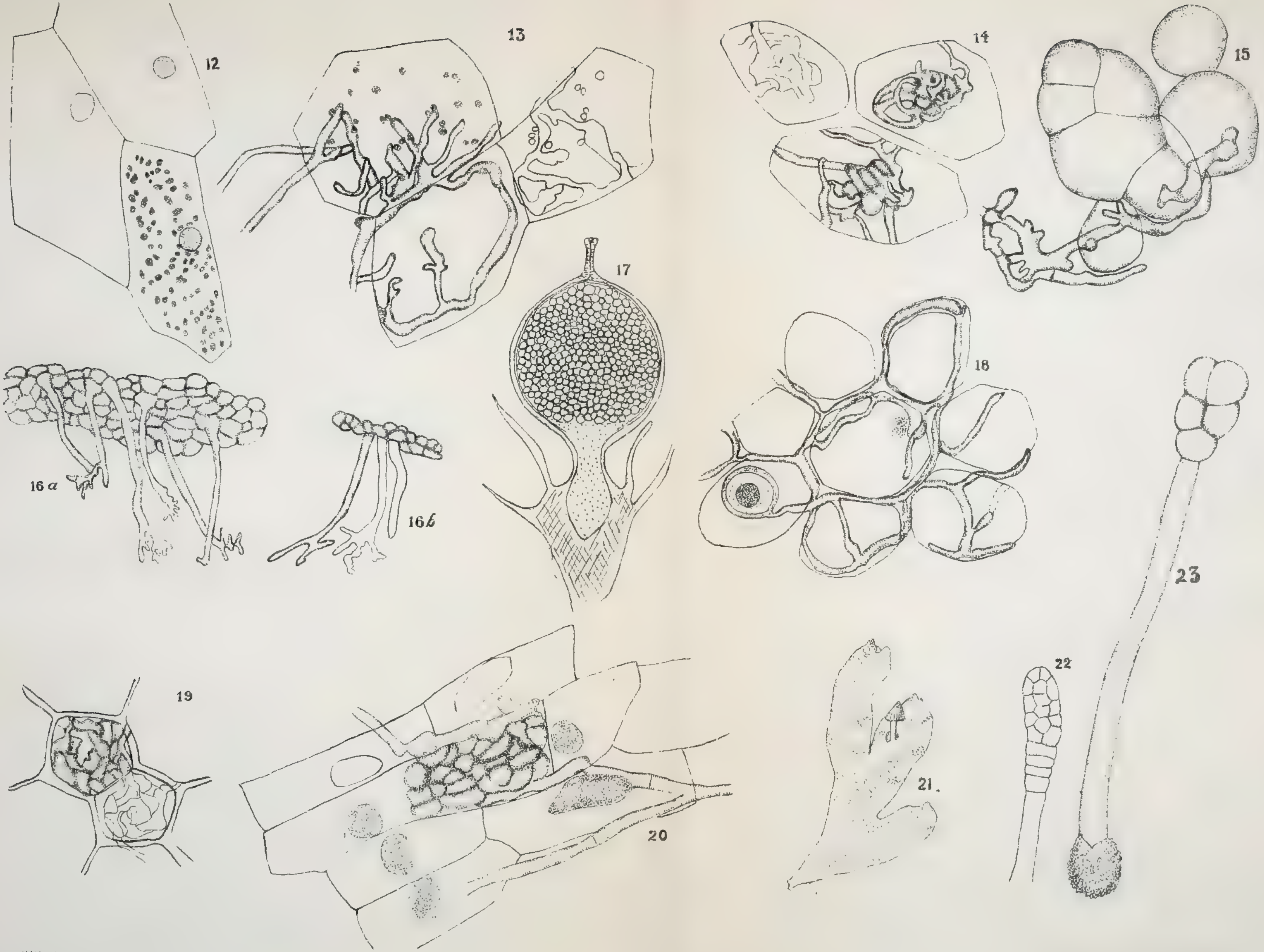

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